Use of T cell function to determine the effect of physiologically active food components

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ABSTRACT  The interdependency between the disciplines of nutrition and immunology was recognized in the 1970s when immunologic measures were introduced as a component of assessing nutritional status. Today, the immune response is considered integral to the pathophysiology of many chronic diseases in which diet plays a major role in prevention or treatment. T lymphocytes are an important adaptive cellular component of the immune system. Because of the difficulty in quantifying and isolating T cell function through clinical measures and in vivo immune challenges, most assessments of the effect of nutrition on immunity have been performed in vitro. A frequently used in vitro method to assess the cell-mediated response to nutritional intervention is lymphocyte blastogenesis. During the past 20 y, many soluble factors (cytokines) that influence cells involved in the immune and inflammatory responses have been described. Changes in dietary fat can modulate cytokine production in the absence of disease. Apoptosis (programmed cell death) is an exciting new area; a decrease in the rate of apoptosis may play a role in the pathogenesis of autoimmune disease and age-related events such as tumorigenesis. Energy restriction increases apoptosis. The goal of studying biomarkers of immune function is to understand how specific nutrients or foods directly and indirectly affect immunity. Biomarkers must be identified that can predict with reasonable accuracy resistance to infection and other illnesses associated with poor immune function. Am J Clin Nutr 2000;71(suppl):1720S–5S.

KEY WORDS  T lymphocyte, immune function, cytokine, apoptosis, gene expression, biomarker

NUTRITION AND IMMUNE FUNCTION  In general, providing extra energy, multiple micronutrients, or moderately large doses of single nutrients improves immune function, and thus nutrition is regarded as an important determinant of the immune response (1). This understanding has developed out of the growth in the field of immunology from a descriptive science to one in which diverse immune phenomena can be coherently tied together and explained in precise structural and biochemical terms. The interdependency between the disciplines of nutrition and immunology was recognized formally in the 1970s when immunologic measures were introduced as part of the assessment of nutritional status (2). Today, protein-energy malnutrition is accepted as a major cause of immunodeficiency worldwide (1), and the immune response is considered integral to the pathophysiology of many chronic diseases in which diet plays a major role in prevention or treatment (3).

Both the nutritional state and specific nutrients may affect the immune system directly (eg, by triggering immune cell activation or altering immune cell interactions) or indirectly (eg, by changing substrates for DNA synthesis, altering energy metabolism, changing physiologic integrity of cells, or altering signals or hormones). Knowledge of the impact of nutritional status on the functioning of the immune system has led to several practical applications, including the use of immunologic tests as prognostic indexes in patients undergoing surgery and the use of immunologic methods to assess nutritional status and to determine the efficacy and adequacy of nutritional therapy (4). In addition, the role of specific nutrients or foods in stimulating the immune response is being explored. New information should permit the development of uniquely designed feeding formulas or regimens with selected ingredients to optimize immune function in specific segments of the general and hospitalized populations. In the critical care area, several specific nutritional supplements have already been designed that are aimed at reducing the risk of infection and improving the recovery of immune function in immunocompromised patients (5).

THE IMMUNE SYSTEM AND T LYMPHOCYTES  The immune system (the cells and molecules responsible for immunity) is defined as part of the host’s defense against destructive forces either from outside the body (eg, bacteria, viruses, and parasites) or from within (eg, malignant and autoreactive cells). Innate (natural) immune defenses are those components of the immune system (macrophages, monocytes, and neutrophils) that...
function without relying on prior exposure to a particular antigen. They are the early phases of the host defense that protect the organism during the 4–5 d it takes for lymphocytes to become activated. Adaptive or acquired immune responses develop over the lifetime of a human being in response to environmental challenges (pathogens and antigens). Lymphocytes are the primary cells of this arm of the acquired immune system.

The T lymphocytes (T cells), which are an important adaptive cellular component of the immune system, can both modulate the function of other immune cells and directly destroy cells infected with intracellular pathogens. During development, each T cell (these cells are derived from hematopoietic stem cells in bone marrow) generates a unique receptor by rearranging its receptor genes, enabling the cell to produce receptors with an almost infinite range of specificities. Once they are mature, T cells migrate from the thymus and perhaps the gut to the periphery, where they encounter antigens presented to them by specialized antigen-presenting cells in the context of a class I or II major histocompatibility complex molecule.

An additional signal (accessory molecule) delivered from B cells, macrophages, or dendritic cells is needed to induce lymphocyte proliferation and differentiation [Figure 1 (6)]. Today, the response by T cells is considered to be not only a factor in acute infections but also an integral component of biological processes such as development and aging as well as the pathophysiology of many chronic diseases (eg, rheumatoid arthritis, type 1 diabetes, celiac disease, cancer, and cardiovascular disease) (3, 7).

MEASURING T CELL FUNCTION

Although there are many ways to assess the immune system of a human or an animal, at present there is no one overall measure of immunity, making it difficult to design and assess studies aimed at determining the effect of a nutrient or food component on immunity. Measuring T cell function focuses on the ability of these cells to be stimulated and secrete activation factors. These responses enable T cells to modulate the inflammatory and humoral immune system, destroy infected and transformed cells, maintain memory, and participate in the delayed-type hypersensitivity (DTH) response and graft rejection. The main paradox facing a researcher working in this field is that nutritional modifications are not likely to influence only one aspect of the immune system, and even if they do, they may alter how the other parts function. This review addresses some of the issues in determining the effects of nutrition on T cell function. Details of the specific techniques that nutritionists can use to measure immune function are published elsewhere (7).

Issues in designing a study

Because there is considerable interindividual variation in immune function, study designs should include measures that are performed both before and during the intervention period to control the variability. As with any biological measurement, the variation observed in the primary outcome measures should be used to calculate the appropriate number of study subjects needed. Changes in physiologic states (eg, from stress, exercise, menstrual cycle, time of the day, and time after last meal) and minor illnesses must be considered in selecting sampling times. For many reasons (ethical and practical as well as the problem of defining and quantifying endpoints), it is not possible to use humans as subjects for such studies, but animal studies can be useful for determining the effects of complex dietary interactions on the immune system. The biological and biochemical diversity of different models can be used for experimental advantage. Long-term intervention trials, of the type used in experimental research in animals, are rarely practical in human populations. Animal research enables one to tightly control diet, examine putative intermediate markers, test hypotheses about mechanisms, and quantify effects in a shorter period. There is no ideal animal model, however, and the limitations to animal studies are recognized.

It has been suggested that strict control of several variables (ie, age, timing of the exposure to the nutrient or food, health status, genetics, past exposure to the immune challenge, tissue selection, and antigen dose) is needed to make observations in animal studies applicable to humans (8). Tissue sampling is particularly important, and caution should be taken when making comparisons between species based on sampling of different tissues. For example, cells isolated from human blood and mouse spleen do not always react the same way to a mitogen (8).

Considerations in design of experimental diets

The interactive effects of other nutrients with the nutrient of interest make it difficult to design a study that controls for all possible immunomodulatory nutrients. In animal and human studies, either a purified or natural ingredient diet is the logical choice for the background diet in which the level of the nutrient or compound of interest can be manipulated. Natural ingredient diets have the advantage of containing food ingredients consumed normally by humans, making these diets particularly useful for assessing the effects of interactions between food constituents as well as the relations among these interactions (9). However, they have the disadvantage that changing only 1 or 2 food components at a time is virtually impossible. As a result, complex interactions between food components cannot be studied (9). Purified diets reduce the number of components that can interact with the test nutrient, but interactions can still occur and should always be considered.

In vivo measures of immune function

The ultimate goal of studies is to determine whether a particular nutrient or food improves immune function. Because immunity is the end result of the responses of many different components of the immune system and the interactions of these components with other systems, an in vivo measure that reflects this complicated relation would be ideal.

The DTH response is a widely used in vivo assay for assessing an individual’s bacterial host defense capability (10). Suppression of the response signals a failure of one or more components of the host defense system (9). In this procedure, a series of antigens (ubiquitous antigens derived from bacterial and fungal products as well as 2,4-dinitrochlorobenzene) are injected intradermally in the forearm and the area of induration is measured at 24 and 48 h. The rationale is that Langerhans cells will present the cutaneously encountered antigens to activated or memory T cells. Anergy (loss of cutaneous hypersensitivity) to skin testing is associated with adverse outcome from infections, burns, or surgical trauma and has been used to predict postoperative complications as well as the severity of various types of malnutrition: protein energy, iron, zinc, and vitamins A, C, and B-6 (2, 10, 11). Because of large interindividual variation in the response, sequential testing in subjects might be a more valuable use of this technique. The assumption is that improvement in the DTH response represents an
increased resistance to infection; a decrease in the response was reported to be strongly associated with sepsis and related mortality in intensive care or trauma patients (12).

Neither the sensitivity nor the value of the DTH response for measuring moderate changes in nutrient intake in healthy persons is clear. Despite decreased function defined by in vitro measures of T cell function, in our own research we did not find that feeding a very-low-energy diet to obese persons for 5 wk altered the DTH response to 6 antigens (13). However, DTH response appears to have been used successfully in a study that evaluated the efficacy of a nutritional supplement in the elderly (4).

Several variables—the number of infections, antibiotic use, hospital stay, and hospital cost—have been used to estimate the effect of nutritional intervention on the immune response in hospitalized patients. Finding an appropriate measure for a healthy, free-living population is more difficult, particularly in the short term. In a few studies, nutrient supplementation of elderly subjects reduced infection-related illness (reviewed in reference 4). Because a humoral response depends on T cell function, vaccine response (primary or secondary immunization) has been used as a biomarker of the effect of nutrient supplementation on immune function (14).

Dietary lipid effects on cell-mediated immune function have been assessed in vivo in animals by measuring their effects on graft-versus-host and host-versus-graft responses (15). By using animal models, it is often possible to directly challenge the immune system with an organism. In one study, the responses of local, systemic, and remote immune organs to an intraperitoneal challenge of *Escherichia coli* were assessed in rats fed by total parenteral or enteral nutrition (16).

In vitro measures

Because of the difficulty in quantifying and isolating T cell function by using clinical measures and in vivo immune challenges, most assessments of nutrition on immunity have been performed in vitro.

Obtaining cells

Immune cells can be obtained from various solid organs and body fluids in both humans and animals. The primary lymphoid organs (thymus and bone marrow) contain both mature and immature cells, although the mature fraction is quite small (5–10% of total cells). The secondary lymph organs include the spleen, lymph nodes, Peyer’s patches, tonsils, skin, gut, and other mucosal-associated lymphoid tissue (salivary glands, bronchi, female and male reproductive tracts, and mammary gland). These organs contain immunocompetent lymphocytes that are physiologically interconnected by the migration of most, if not all, lymphocytes. Despite an extensive recirculation system, immune cells isolated from different body sites have been shown to respond differently to diet (17). One of the most obvious and probably most important sites for interactions among macrophages, lymphocytes, and food antigens is the gut-associated lymphoid tissue, where intestinal microflora are also a source of antigenic stimulation. We found that including fermentable fiber in the diet significantly improved the mitogenic response of mesenteric lymph node and intraepithelial lymphocytes in adult dogs (18).

In humans, studies of nutritional effects on immunity most often concentrate on peripheral blood lymphocytes. Automated peripheral white blood cell counts and cell differentials are routine clinical measures that have been used as indexes of protein-energy malnutrition (19). In one study, feeding a very-low-energy reducing diet for 2 wk significantly decreased the concentration of peripheral blood total leukocytes, neutrophils, lymphocytes, and monocytes (13). Caution must be taken, however, when using blood responses to predict immune changes in the secondary lymphoid organs, where the acquired immune response primarily occurs. For example, we found that short-term (2 wk) effects of dietary fiber on the gut-associated immune system in the dog are not found in peripheral blood lymphocytes (18, 20).

Identifying cell types

Immune cells constitute a heterogeneous population, and many methods (eg, panning, adherent columns, complement-mediated lysis, flow cytometry sorting, rosetting, and the use of immunomagnetic particles) are available to purify or enrich specific populations (7). Although useful for mechanistic studies, specific immune cell types generally do not function the same when separated.

Lymphocytes, although heterogeneous in their function, have similar morphologic and physical characteristics. The availability

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**FIGURE 1. T cell activation.** Adapted from Nossal (6). MHC, major histocompatibility complex.
of monoclonal antibodies and the technology of immunofluorescence have made it possible to further separate and classify lymphocytes. The automation of immunofluorescence analysis via flow cytometry allows for the simultaneous measurement of multiple single-cell characteristics (size, granularity, fluorescence) of 500–4000 cells/s as they move through a fluid stream (7). By using multiple fluorochrome-labeled molecules, up to 3 characteristics can be studied simultaneously. For example, 3-color immunofluorescence was used to detect an increase in the relative percentage of immature (CD2+ CD4+ CD8+ cells) using multiple single-cell characteristics (size, granularity, fluorescence) have made it possible to further separate and classify lymphocytes. The automation of immunofluorescence analysis via flow cytometry allows for the simultaneous measurement of multiple single-cell characteristics (size, granularity, fluorescence) of 500–4000 cells/s as they move through a fluid stream (7). By using multiple fluorochrome-labeled molecules, up to 3 characteristics can be studied simultaneously. For example, 3-color immunofluorescence was used to detect an increase in the relative percentage of immature (CD2+ CD4+ CD8+ T cells) in the blood of moderately malnourished elderly subjects (21).

Estimating response to immune challenges

By permitting more comprehensive studies and by collecting reproducible data, studies in cultured cells (immortalized cell lines) have been particularly useful for assessing the mechanisms by which dietary nutrients affect immune function. For example, cultured cell lines have been used to establish several mechanisms for the effects of dietary fatty acids on immune function (22). More often, however, primary cultures (cells obtained from the subjects) are used to determine the cellular response to in vivo dietary exposure. The use of primary cultures avoids the genetic changes that enable cultured cells to survive for long periods. A principal problem with all cell culture studies is determining the conditions that best represent (it is not possible to mimic) the in vivo environment. Culture media are designed to improve cell growth, maintain a cellular phenotype, or promote a phenotypic change. Once the cells are placed in artificial media and serum, their environment, cell concentration or contact, and hormonal or molecular (eicosanoid and cytokine) milieu have been changed. Most important, the nutrition of the cell is now controlled. Sera and media contain nutrients and other growth factors that influence the metabolism and function of cells. Despite these limitations, the use of primary cell cultures enables the standardizing of environmental conditions within and between experiments.

Determining cell-mediated immune function

A frequently used in vitro method for assessing the cell-mediated response to nutritional intervention is lymphocyte blastogenesis (cellular proliferation). Isolated immune cells are incubated with and without stimuli (eg, mitogen, antigen, monoclonal antibody, cytokine, hormone, or any mixture of these). Depending on the stimuli used, information on the interactions among T cells, B cells, and adherent cells can be obtained. Cellular proliferation is commonly estimated by measuring the uptake of labeled compounds such as thymidine. For example, we found that changing the ratio of n-6 to n-3 fatty acids in the rat diet significantly altered the incorporation of [3H]thymidine by mesenteric lymph nodes (Figure 2). In a study of primates, varying the content of n-3 and n-6 fatty acids in the diet increased the response of peripheral blood to a T cell mitogen (23). Alternatively, the expression of activation markers and other proteins on the cell surface in response to stimulation can be used as markers of stimulation. We found that feeding a diet containing 5% (by wt) fish oil to sedentary rats increased the proportion of CD4+ and CD8+ cells expressing the transferrin receptor (CD71) after stimulation with concanavalin A (24). Other possible in vitro measurements of cell-mediated immune function are directed against allogenic histocompatibility antigens such as mixed leukocyte cultures.

Cytokines

During the past 20 y, an astonishing number of soluble factors that influence cells involved in the immune and inflammatory responses have been described. These nonantibody glycoproteins, generated by activated lymphocytes, act as intracellular mediators of the immunologic response and are grouped under the umbrella term cytokines. In vitro, significant information has accumulated regarding the function of the various cytokines alone and together (3), but their physiologic roles in vivo are not as clear. Both nutritional status and specific nutrients have been reported to affect the concentration and production of cytokines (25). For many years, cytokine production was associated only with infection; little credence was given to the fact that some types of nutrients and food could induce cytokine production in healthy subjects. It has now been clearly shown that changes in dietary fat can modulate cytokine production in the absence of disease (25, 26).

Apoptosis and cell cycling

Assessing when a cell has irrevocably died is difficult. Measuring apoptosis (programmed cell death) is a new and exciting area of research. Apoptosis is characterized morphologically by increased cytoplasmic granularity, cell shrinkage, and nuclear condensation. The most prominent feature of apoptosis is the activation of an endogenous endonuclease that degrades nuclear DNA at linker sections to fragments (27). It has been suggested that a decrease in the rate of apoptosis plays a role in the pathogenesis of autoimmune diseases and age-related events such as tumorigenesis. Energy restriction increases apoptosis, which may be the mechanism for its effect in suppressing tumors, ameliorating autoimmune diseases, and prolonging life span (27). Programmed cell death is an endpoint for many cellular events, but it has not been examined in nutrition studies.

Gene expression

It is now well established that specific nutrients can affect gene expression and that these changes significantly affect cellular function (28). In immune cells, nutrients can have an impact on the early signals for gene expression and on the message and proteins of genes such as activation markers and cytokine-activation markers (24).
Metabolism

The use of energy substrates by immune cells is a new and exciting field of research. Glucose and glutamine are the major energy sources for cells of the immune system (17, 29, 30). When immune cells are activated, use of these nutrient substrates increases substantially and immune cells are extremely sensitive to changes in substrate availability (29). Thus, lymphocyte metabolism is a possible biomarker for many nutritional interventions. In a comparison with a nonpurified laboratory diet, we showed that feeding a purified casein-based diet to BB diabetes-prone rats altered the rate of production of the major end products of glutamine and glucose in cells from lymphoid organs (17). Because of the importance of glutamine to immune cells, glutamine uptake has been proposed as an early marker of lymphocyte activation (30). The immunostimulator effects of other nutrients such as nucleotides and arginine have been attributed to their roles as substrates to support the high rates of DNA and RNA synthesis during immune activation or as precursors for nitric oxide production.

Membrane composition

In lymphocytes, events associated with the plasma membrane play an important role in signal transduction, the expression of surface-associated molecules, enzymatic activities, and cellular activation (31). In other cell types, modifying the lipid composition of the plasma membrane alters its function (32). Changing the fatty acid composition of lymphocyte phospholipids may be the mechanism by which fatty acids modulate lymphocyte functions (22). Changing the n–6 and n–3 content of immune cell lipids affects lymphocyte proliferation, cell-to-cell adhesion, plasma membrane fluidity, the activity of membrane-bound enzymes, cytokine production, and the expression of some activation epitopes (31, 33). Feeding a diet containing 5% (by wt) long-chain n–3 fatty acids (as fish oil containing 46% 20:5n–3, 4% 22:5n–3, and 16% 22:6n–3) to rats significantly increased the n–3 content and decreased the n–6 fatty acid content of the major membrane phospholipids in the lymphocyte plasma membrane (Figure 3). These membrane changes were associated with changes in estimated rates of proliferation (Figure 2).

CONCLUSIONS

Ultimately, the goal of studying biomarkers of immune function is to understand how specific nutrients or foods directly or indirectly affect immunity. At present, there are notable gaps in our knowledge of the connections between in vitro responses and in vivo realities. The consequences of changes in many in vitro measures made in healthy humans or animals still require testing to understand their relation to health. The task at present is to define biomarkers (an immunologic index or indexes) that can predict resistance to infection and other illnesses associated with poor immune function with reasonable accuracy. Many of the biological measures currently used as indicators of immune function show promise in this respect. For example, elevation of immune responsiveness (as measured by in vitro proliferation) through nutrient supplementation was reported to correlate with increased resistance to infection in older persons (8). In this study, an inverse relation between the DTH response to 5 antigens and mortality during the preceding 2 y was found among 52 subjects aged > 80 y (8). Unfortunately, much of the work on establishing biomarkers has been performed with human malnutrition, which is usually a composite syndrome of multiple nutrient deficiencies (4). To further complicate this issue, one cannot assume that results of short-term feeding studies can be directly applied to what would be observed in chronic feeding studies.

There is growing interest in identifying the roles of specific nutrients in optimizing immune function. At present, we have no single biomarker or gold standard that can be used to determine the biological relevance of a novel measure of immunity. Perhaps the best approach is to attempt to include in a study both an in vivo endpoint and multiple measures of a particular aspect of T cell function. Doing this will help answer definitively how specific nutrients and foods modulate immune function.
REFERENCES